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ALLOZYME VARIABILITY WITHIN AND AMONG VARIETIES OF *ISOMERIS ARBOREA* (CAPPARACEAE)

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Abstract

The pattern of genetic variation within and among 13 natural populations (294 individuals) representing the four varieties of *Isomeris arborea* (Capparaceae) throughout its range in California was investigated with allozyme electrophoresis. Thirty enzyme loci were examined. Low genetic diversity within populations ($A = 1.18$, $P = 0.139$, $H_E = 0.051$ and $H_O = 0.051$) and significant genetic differentiation among populations ($F_{ST} = 0.462$) were found. A relatively large number of private alleles (9) were detected as well as several unique alleles confined to single varieties. We found a relatively high proportion of total genetic diversity (45%) among varieties and a significant correlation ($P < 0.001$) between F_{ST} and geographic distance. Multiple regression analyses demonstrated clines in genetic diversity measures, from East to West and South to North. Multiple Correspondence Analysis (MCA) clearly demonstrated division into a minimum of two groups. The above observations together with low gene flow estimates suggest genetic drift by isolation may have been critical to the current genetic structure of the species. We tentatively argue, based on our results, that variety *globosa* may be deserving of subspecies status.

Key Words: *Isomeris arborea,* genetic structure, Capparaceae, gene flow, bladder pod.

Almost all species exhibit some degree of spatial genetic heterogeneity (Avise 1994). While spatial genetic structure can result from several processes, in plant species the amount of gene flow is pivotal in determining the distribution of genetic variation (Loveless and Hamrick 1984; Hamrick and Godt 1990). Gene flow in plant species can be highly localized (Endler 1977) as well as temporally and spatially variable (Govindaraju 1989; Ellstrand 1992). Restriction of gene flow by limited seed and/or pollen dispersal might produce isolation by distance with subsequent differentiation among groups by genetic drift. Spatial genetic structure can also reflect an interaction between environmental factors that are spatially and/or temporally dynamic and various life-history characteristics (Slatkin 1985; Hamrick and Godt 1990, 1996; Boyle et al. 1990; Perry and Knowles 1991; Knowles et al. 1992). For example, the interaction of reproductive dynamics and disturbance (decreased fire frequency intervals) may account for some of the genetic structure found in *Cupressus forbesii* (Truesdale and McClenaghan 1998). The distribution of genotypes within and among populations may also be affected by selection with patterns of differentiation reflecting spatially varying selective regimes. Species which occupy an array of habitats among which dispersal is limited may exhibit local adaptation (Slatkin 1985) and isolation-by-distance even in the absence of spatially varying selection (Wright 1951; Endler 1977).

Isomeris arborea Nutt. (=*Cleome isomeris* E. Greene; Capparaceae) is a drought-deciduous, perennial shrub endemic to southern California and Baja California, Mexico, where it grows in several habitats from the coast to the desert. Commonly called bladderpod, *I. arborea* is a monotypic genus (Vanderpool 1993) consisting of four varieties: *I. arborea* var. *arborea* (coastal), *I. arborea* var. *angustata* Parish (desert), *I. arborea* var. *globosa* Cov. (Tehachapi Mountains) and *I. arborea* var. *isularis* Jepson (southern Channel Islands). These varieties, hereafter referred to as *arborea, angustata, globosa* and *insularis,* are recognized primarily on the basis of fruit morphology, which appears to be loosely correlated with geography (Vanderpool 1993; Truesdale personal observation). Fruits are dehiscent, photosynthetic, contain a conspicuous inner gas space, and contribute significantly to the carbon economy of the plant and developing seed (Goldstein et al. 1990). *Isomeris arborea* is andromonoecious, producing both male and hermaphroditic flowers on the same plant. Flower visitation by insects is high (Truesdale personal observation). Little recruitment within populations is observable but may occur episodically. Seeds are heavily preyed upon by a variety of rodent species (Niccoli 1987), are relatively heavy and not readily dispersed. The species is not a true xerophyte, being unable to tolerate the low soil water potentials typically found

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in the desert, but is apparently able to access perched ground water under sandy washes and depressions with its deep, rapidly growing tap-root system (Goldstein et al. 1990).

Stebbins and Major (1965) have suggested that *I. arborea* was a member of the Madro-Tertiary Geoflora and list it as a paleoendemic. Iltis (1957) places *Isomeris* in *Cleome* sect. peritoma based on morphological characteristics. While the geographic origin of *Cleome* is unknown, Axelrod (1948) suggests that the important distribution center was the Mexican highlands. The overall distribution of the genus *Cleome* parallels the present-day distribution of the Madro-Tertiary flora, is most probably of mesophytic origin and is proposed as having initially developed pre-Eocene (Axelrod 1958; Raven and Axelrod 1978). The northward migration of the Madrean vegetation from southwestern North America, which began during the Pleiocene, and radiated northwestwardly into semi-arid areas of the western United States may account for some of the differentiation found in the genus (Raven and Axelrod 1978). During the late Pleiocene, orogenic activity led to formation of the Sierra, Transverse and Coastal Ranges as well as the formation of the Great Basin and Southwestern deserts, and the current desert flora of southern California developed from the Madro-Tertiary Geoflora (Axelrod 1948, 1958). The distribution of modern desert species is proposed by Raven and Axelrod (1978) to have been primarily shaped by the extreme topographic, edaphic and climatic diversity associated with fluctuations in climate in conjunction with dynamic geologic change. The spread of warmer, drier climate since the Xerothermic enabled taxa existing in semi-desert sites to spread to the southern California coast and the Channel Islands (Raven and Axelrod 1978).

Johnson (1968), in contrast, has proposed that *I. arborea* was a desert colonizer, with coastal populations being much older than desert populations and pre-adapted to dry habitats, having developed in a much earlier, much drier coastal environment. As deserts formed in the late Pleistocene, *I. arborea* was able to invade and survive the arid conditions as a result of pre-adaptations.

Gittins (1965) has proposed that the formation of the Transverse Ranges separated *globosa* from the desert variety and allowed the fixation of the extreme globose form of seed capsule in this area through isolation and subsequent genetic drift. Variety *insularis* occurs naturally only on the Southern Channel Islands, which are of Mexican origin (Schoenherr 1992).

We had several goals in this study. We wanted to describe the population genetic structure among *I. arborea* populations. With the exception of *globosa* there is little morphological divergence among varieties. This suggests that 1) the region, as a whole, has been more recently colonized than proposed and populations have not had sufficient time to develop unique traits that would warrant species designation, 2) the species is a paleoendemic but sufficient gene flow exists to maintain genetic and phenotypic cohesiveness or alternatively, 3) significant genetic divergence among populations may exist that does not correlate with phenotypic divergence. When morphological variation contains little information as in *I. arborea,* molecular evidence is particularly useful in illuminating population structure. We also wanted to ascertain whether a cline of reduced genetic variability along one or the other putative paths of colonization could be detected as current genetic structure may reflect past migration in organisms with relatively long generation times (Newton et al. 1999). In addition, Gittens' (1965) hypothesis regarding the origin of *globosa* through isolation and subsequent genetic drift suggested that genetic variation in *globosa* should be significantly lower than in other varieties.

Materials and Methods

Leaf tissue representing the four varieties of *Isomeris arborea* was collected from 13 localities within California (Table 1). Populations sampled represent a mixture of localities originally sampled by Gittens (1965) which were still extant, and populations used in other studies by one of us (BDC). Tissue was transported to the lab on ice, stored at 4°C and extracted within 24 hours of collection. Approximately 0.5 g of leaf tissue was homogenized in an 1.5 ml Eppendorf tube with freshly prepared 0.2 M phosphate buffer (pH 7.5) containing 0.20 M sodium tetraborate, 0.01 M sodium metabisulfite, 0.015 M diethyldithiocarbamic acid sodium salt, 2% (w/v) L-ascorbic acid sodium salt, 2% (w/v) PVP-40, 0.5% (v/v) 2-mercaptoethanol and 10 mg/25 ml NADP.

Crude extracts were absorbed onto filter paper wicks and placed into 12% horizontal starch gels composed of the following buffers: lithium hydroxide: pH 8.3, histidine-citrate: pH 6.2, and tris-versene-borate: pH 8.6 (May 1994). A total of 30 loci could be reliably scored of which 10 were polymorphic: non-enzymatic general protein (GP 3); β-esterase (Est, E.C. 3.1.1.1); glucose-6-phosphate isomerase (Pgi 2, E.C. 5.3.1.9); isocitrate dehydrogenase (Idh, E.C. 1.1.1.42); malic enzyme (Me, E.C. 1.1.1.40); menadione reductase (Mnr 1,2, E.C. 1.8.1.4); peroxidase (Per 2, E.C. 5.3.1.9); phosphogluconate dehydrogenase (6Pgd 2, E.C. 1.1.1.44); phosphoglucomutase (Pgm 1, E.C. 5.4.2.2.

The largest sample (Monument Road) was chosen as the reference population due to the large number of alleles (8) present at the β-esterase locus, and individuals of known electrophoretic mobility from this population were included on all gels to facilitate scoring.

Allele frequencies were calculated using Biosys-1 (Swofford and Selander 1981). Genetic variability parameters estimated included the mean number of

TABLE 1. LOCATION DATA: POPULATION NAME, POPULATION DESIGNATION, ALTITUDE AND SAMPLE SIZE FOR THE 13 POPULATIONS ANALYZED FOR GENETIC VARIATION IN *ISOMERIS ARBOREA*. Population designations refer to capsule type: A = *arborea*; G = *globosa*; T = *angustata*; I = *insularis*.

Population name	Population designation	Location lat, long	Altitude (m)	n
Monument Road	A1	32°32.54′N, 117°06.28′W	13	30
Otay Mesa	A2	32°32.88′N, 116°59.40′W	60	28
Sorrento Valley	A3	32°54.07′N, 117°13.18′W	15	20
Christianitos Road	A4	33°24.12′N, 117°35.39′W	280	20
Gorman	G1	34°47.59′N, 118°50.56′W	1228	20
Oildale	G2	35°31.95′N, 118°58.32′W	327	20
Cache Creek	G3	35°07.84′N, 118°13.96′W	1111	20
Caliente Creek Road	G4	35°17.22′N, 118°37.56′W	486	20
Mecca	T1	33°36.45′N, 115°50.06′W	305	25
Cabazon	T2	33°55.10′N, 116°45.50′W	500	28
Amboy	T3	34°34.69′N, 115°50.02′W	415	20
Joshua Tree	T4	33°43.25′N, 115°49.52′W	920	21
San Clemente Island	I1	33°00.54′N, 118°23.28′W	314	22

alleles per locus (A), percentage polymorphic loci (P), the observed heterozygosity (H_O) and expected heterozygosity (H_E). Conformity to Hardy-Weinberg expectations was determined by the exact test proposed by Guo and Thompson (1992) with the overall significance for each locus estimated by Fisher's combined probability test (Fisher 1970) using the GENEPOP program (version 2; Raymond and Rousset 1995). Levels of intervarietal divergence in these measures were compared by unpaired two-tailed t-tests using a Welch-Satterthwaite correction (homogeneity of variance not assumed).

Unbiased estimates of Wright's F-statistics were calculated according to the methods of Weir and Cockerham (1984) using FSTAT (version 2.9.1; Goudet 2000). F_{IS} and F_{IT} measure the correlations between two uniting gametes relative to the subpopulation and total population respectively, while F_{ST} measures the correlation between two gametes randomly drawn from subpopulations and is a measure of the degree of genetic differentiation of subpopulations. Standard errors for single locus estimates of F-statistics were calculated by jackknifing over populations, and 95% confidence intervals of mutilocus estimates were calculated by bootstrapping over loci (Weir 1990). The significance of population differentiation was estimated by permuting genotypes among samples using 15,000 randomizations of the data. Wherever necessary, the significance level of each analysis was adjusted by taking into account the number of multiple tests of the same hypothesis using the sequential Bonferroni method (Holm 1979). Values of gene diversity parameters (Nei and Chesser 1983) were also calculated using FSTAT and hierarchically subdivided (Chakraborty 1980) to allow comparison to other studies.

The private alleles method (Slatkin 1985; Slatkin and Barton 1989) was used to estimate gene flow. Differentiation among subpopulations, F_{ST}, is produced by genetic drift, and countered by gene flow. Isolation by distance was analyzed by regressing pairwise estimates of F_{ST} vs. geographic distance among populations. Statistical significance was determined by Mantel analyses (Mantel 1967) using randomization testing (10,000 randomizations). Multiple regression analyses were performed on population level genetic diversity estimates using latitude, longitude and elevation as independent variables. Genetic diversity estimates (dependent variables) representing proportions (H_E, P) were arcsine transformed prior to analysis.

We also carried out a multiple correspondence analysis (MCA) with the Ecological Data Analysis software package (ADE 4; Guinand 1996), which allowed the data for individual populations to be viewed on a general factorial plane. This ordination technique, which measures correlations between the presence and absence of alleles within a contingency table, was utilized to analyze the data for the existence of complex spatial geographic clusters. The ADE 4 package was also used to determine the contribution of each axis to the total variance.

RESULTS

Of the 30 putative loci scored in this study, 10 (33%) were polymorphic in at least one population (Table 2). Of the 31 alleles detected, 9 (29%) were restricted to single populations at relatively high frequencies (mean = 0.272, range 0.100 to 0.614). Furthermore, several allelic variants were confined to a single variety, though none of these were fixed. There was no significant departure from Hardy-Weinberg expectations at any locus or significant correlation with sample size for any genetic measure.

Average number of alleles per locus (A) ranged from 1.07 at A3 to 1.30 at T1 and T3, percentage of polymorphic loci (P) from 6.67% (A3) to 20.00% (T1, T2, T4) and gene diversity (H_E) from

TABLE 2. ALLELE FREQUENCIES AT 10 POLYMORPHIC LOCI IN *ISOMERIS ARBOREA* POPULATIONS. Population designations are given in Table 1. Frequencies in bold text with underscoring indicate private alleles. Those confined to one varietal type are in italics and bold text.

		Population												
Locus	Allele	A1	A2	A3	A4	G1	G2	G3	G4	T1	T2	T3	T4	I1
Est B	1.27	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.405**	0.000
	1.15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.341**
	1.09	0.000	**0.304**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	1.00	0.350	0.393	0.475	0.325	1.000	1.000	1.000	1.000	0.660	0.679	0.675	0.214	0.525
	0.94	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.190**	0.000
	0.85	0.550	0.304	0.525	0.675	0.000	0.000	0.000	0.000	0.200	0.214	0.275	0.143	0.114
	0.79	**0.100**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.70	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	***0.140***	***0.107***	***0.050***	***0.048***	0.000
Per 2	1.00	1.000	1.000	1.000	0.825	0.075	0.000	0.000	0.000	0.620	0.593	0.525	0.548	1.000
	0.90	0.000	0.000	0.000	0.175	0.925	1.000	1.000	1.000	0.360	0.357	0.350	0.405	0.000
	0.75	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	***0.020***	***0.050***	***0.125***	***0.048***	0.000
Pgi 2	1.13	0.000	0.000	0.000	0.000	0.550	0.600	0.425	0.700	0.020	0.018	0.000	0.024	0.000
	1.00	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.980	0.982	1.000	0.976	1.000
	0.75	0.000	0.000	0.000	0.000	***0.450***	***0.400***	***0.575***	***0.300***	0.000	0.000	0.000	0.000	0.000
Pgm 1	1.00	0.983	1.000	1.000	1.000	0.500	0.575	0.650	0.850	0.980	0.982	0.925	0.976	1.000
	0.93	0.017	0.000	0.000	0.000	0.450	0.400	0.325	0.100	0.020	0.018	0.075	0.024	0.000
	0.84	0.000	0.000	0.000	0.000	***0.050***	***0.025***	***0.025***	***0.050***	0.000	0.000	0.000	0.000	0.000
Mr 1	1.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.773
	0.88	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.227**
Mr 2	1.00	1.000	0.821	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.50	0.000	**0.179**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Idh	1.10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.614**
	1.00	0.950	0.946	1.000	1.000	0.900	0.500	0.450	0.450	0.120	0.679	0.200	0.095	0.273
	0.80	0.050	0.054	0.000	0.000	0.100	0.500	0.550	0.550	0.880	0.321	0.800	0.905	0.114
Gp 3	1.17	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.060	0.071	0.000	0.405	0.000
	1.00	0.750	1.000	1.000	0.100	1.000	1.000	1.000	1.000	0.600	0.607	0.550	0.595	0.568
	0.80	0.250	0.000	0.000	0.900	0.000	0.000	0.000	0.000	0.340	0.321	0.450	0.000	0.432
6-Pgd	1.00	1.000	1.000	1.000	1.000	1.000	1.000	0.875	1.000	1.000	1.000	1.000	1.000	1.000
	0.90	0.000	0.000	0.000	0.00	0.000	0.000	**0.125**	0.000	0.000	0.000	0.000	0.000	0.000
Me	1.00	1.000	1.000	0.925	1.000	1.000	1.000	1.000	0.725	1.000	1.000	1.000	1.000	1.000
	0.82	0.000	0.000	0.075	0.000	0.000	0.000	0.000	0.275	0.000	0.000	0.000	0.000	0.000

0.022 at A3 to 0.068 at T4 (Table 3). Gene diversity (H_E) was significantly lower in *arborea* than *globosa* (t = −4.8167, P = 0.0044) or *angustata* (t = −9.0811, P = 0.00079). For the measures A and P, *arborea* was significantly lower than *angustata* (P = 0.0036 and 0.0022 respectively) but comparisons to *globosa* were non-significant (P = 0.24 and 0.18 respectively).

The mean value of F_{ST} over all loci and populations is 0.462, indicating substantial genetic differentiation (Table 4). Allele frequencies differ significantly among populations by an exact test ($P < 0.0001$), and multilocus genotypes differed markedly among varieties. Intravarietal F_{ST} estimates were significant ($P < 0.01$) in *arborea* (F_{ST} = 0.285) and *angustata* (F_{ST} = 0.098), but not *globosa* (F_{ST} = 0.082). Permutation testing of pairwise estimates of F_{ST} within varieties displayed similar significance.

Average gene diversity values were: H_T = 0.276, H_S = 0.152, D_{ST} = 0.124 and G_{ST} = 0.449. Hierarchical analysis revealed that D_{ST} was divided into D_{SV} (gene diversity among populations within varieties) = 0.021 and D_{VT} (gene diversity among varieties) = 0.103. Therefore, 7.5% of the total genetic diversity could be accounted for by variation among populations within varieties, 37.4% by variation among varieties and 55.1% by variation within populations.

The estimate of Nm was 0.125 from the private alleles method of Slatkin (1985). Intravarietal estimates were consistent with the above for *arborea* (Nm = 0.388), but inconsistent for *angustata* and *globosa* (both Nm > 1.0). Mantel analysis between pairwise F_{ST} values and geographic distances (km) revealed that a clear association exists between these two variables ($P < 0.001$ for 10,000 randomizations).

Multiple regression analysis on the 12 mainland populations of *I. arborea* revealed that gene diversity (H_E) was significantly correlated with both latitude and longitude. A significant decline in population levels of diversity was indicated from east-to-west, and a significant increase from south-to-north (Table 5). Mean number of alleles per locus and percentage polymorphic loci displayed the same significant association for longitude but not latitude. No dependent variable was correlated with elevation.

TABLE 3. GENETIC VARIABILITY MEASURES: OBSERVED HETEROZYGOSITY (H_O); GENE DIVERSITY (H_E); MEAN NUMBER OF ALLELES PER LOCUS (A); PERCENT POLYMORPHIC LOCI (P) AND SAMPLE SIZE (N) FOR *ISOMERIS ARBOREA* POPULATIONS. All estimates of variability measures are based on all 30 loci scored with standard errors in parentheses. Means for variability measures are weighted with standard deviations in parentheses. Population abbreviations are given in Table 1.

Population	H_O	H_E	A	P (99%)	n
A1	0.036 (0.022)	0.036 (0.023)	1.17 (0.08)	13.33	30
A2	0.035 (0.024)	0.036 (0.024)	1.13 (0.08)	10.00	28
A3	0.021 (0.017)	0.022 (0.018)	1.07 (0.05)	6.67	20
A4	0.030 (0.018)	0.031 (0.018)	1.10 (0.06)	10.00	20
G1	0.045 (0.025)	0.046 (0.025)	1.17 (0.08)	13.33	20
G2	0.050 (0.028)	0.051 (0.028)	1.13 (0.08)	10.00	20
G3	0.056 (0.028)	0.057 (0.028)	1.17 (0.08)	13.33	20
G4	0.050 (0.025)	0.051 (0.026)	1.13 (0.06)	13.33	20
T1	0.060 (0.028)	0.061 (0.029)	1.30 (0.12)	20.00	25
T2	0.066 (0.030)	0.067 (0.030)	1.27 (0.11)	20.00	28
T3	0.065 (0.029)	0.066 (0.030)	1.20 (0.09)	16.67	21
T4	0.066 (0.033)	0.068 (0.033)	1.30 (0.13)	20.00	21
I1	0.065 (0.031)	0.067 (0.032)	1.20 (0.10)	13.33	22
Means	0.050 (0.015)	0.051 (0.015)	1.18 (0.07)	13.85	23

Axis 1 of the MCA projection (Fig. 1) clearly distinguishes *globosa* from other varieties and explains approximately 62% of the variance in allele frequencies. Three clusters related to pod type are indicated: the first consists of all *globosa* populations, the second of all *arborea* populations and the third of *angustata* populations T1, T2, and T3. Population T4 contains two private alleles and differs markedly in frequency from other *angustata* populations at several loci. Population I1 contains three unique alleles at high frequencies (range: 0.614–0.227).

DISCUSSION

Our results show a significant reduction in gene diversity and number of alleles in *arborea* (coastal) populations. Widespread loss of alleles and increased homozygosity is a general expectation from repeated founder events along a path of range expansion (Hewitt 1996, 1999). Multiple regression analyses indicate significant east-to-west and south-to-north clines in genetic diversity measures. All measures of variation show *angustata* populations to be genetically more diverse compared to *arborea* and *globosa* populations.

In contrast, we detected a number of private alleles at high frequency and unique alleles confined to single varietal types. Furthermore, there is relative intravarietal homogeneity but high intervarietal differentiation. This indicates these varieties have been genetically separated for a substantial period of time, implies that this species may now be in the process of speciation and is characteristic of relict populations (Slatkin 1993).

We conclude that these patterns are more supportive of Axelrod's (1948, 1958) interpretation of the history and development of the Madro-Tertiary Flora as displaying a radial pattern of spread over southwestern North America in response to climatic and geologic change. As orogenic activity and

TABLE 4. F-STATISTICS (WEIR AND COCKERHAM 1984) FOR TEN ELECTROPHORETIC LOCI SURVEYED IN 13 POPULATIONS OF *ISOMERIS ARBOREA* FROM CALIFORNIA. Standard errors of the jackknifed estimates over populations are given in parentheses. Means are jackknifed estimates over loci with 95% bootstrap confidence intervals in parentheses below. Significance of population differentiation was determined by 15,000 randomizations of genotypes among samples. *** $P < 0.0001$.

Locus	F_{IS}	F_{IT}	F_{ST}
Est β	0.123 (0.041)	0.357 (0.053)	0.267 (0.059)***
Per 2	0.043 (0.068)	0.643 (0.143)	0.625 (0.141)***
Pgi 2	0.086 (0.089)	0.713 (0.040)	0.686 (0.028)***
Pgm 1	0.017 (0.166)	0.281 (0.096)	0.275 (0.053)***
Mr 1	0.283 (0.136)	0.479 (0.229)	0.229 (0.109)***
Mr 2	0.105 (0.113)	0.549 (0.114)	0.492 (0.090)***
Idh	−0.102 (0.066)	0.314 (0.190)	0.373 (0.148)***
Gp 3	−0.013 (0.088)	0.201 (0.155)	0.211 (0.109)***
6-Pgd	0.643 (0.309)	0.770 (0.370)	0.191 (0.092)***
Me	0.183 (0.151)	0.467 (0.276)	0.307 (0.151)***
Mean	0.062	0.495	0.462***
	(−0.013–0.111)	(0.342–0.608)	(0.314–0.580)

TABLE 5. MULTIPLE REGRESSION ANALYSIS OF LATITUDE, LONGITUDE AND ELEVATION ON EXPECTED HETEROZYGOSITY (H_E), MEAN NUMBER OF ALLELES PER LOCUS (A) AND PERCENTAGE POLYMORPHIC LOCI (P) FOR 12 MAINLAND *ISOMERIS ARBOREA* POPULATIONS. The r^2 values provided are adjusted for sample size.

Variable	Slope	SE	t	P
Dependent variable = Expected heterozygosity (r^2 = 0.775)				
Latitude	1.497	0.391	3.824	0.005
Longitude	−1.393	0.321	−4.325	0.003
Elevation	0.001	0.001	1.285	0.235
Constant	124.594	34.069	3.657	0.005
Dependent variable = Mean number of alleles per locus (r^2 = 0.640)				
Latitude	0.012	0.021	0.570	0.584
Longitude	−0.051	0.015	−3.936	0.010
Elevation	0.050	0.031	0.447	0.142
Constant	7.165	1.531	4.670	0.005
Dependent variable = Percentage polymorphic loci (r^2 = 0.623)				
Latitude	1.248	0.888	1.406	0.197
Longitude	−2.718	0.727	−3.739	0.006
Elevation	0.003	0.002	1.562	0.157
Constant	296.514	77.244	3.839	0.006

marked seismic events led to a diversification of topography, new, more localized habitats developed in the southwest (Axelrod 1948, 1958). Dramatically fluctuating Pleistocene climate changes impacted these environments (Axelrod 1948). The influence of these highly variable, narrowly localized environments continued into the Holocene (Cole and Wahl 2000) and may explain some of the diversification found in *I. arborea.*

Our results also show a strong pattern of increasing F_{ST} with increasing geographic distance among the populations studied and a high degree of genetic divergence among populations regionally. Gene flow estimates from private alleles (Nm = 0.125) indicate that the number of migrants per generation is insufficient to preserve genetic cohesiveness. Furthermore, hierarchical analysis of population subdivision indicated that more cohesiveness exists within rather than between varieties, and that varieties were differentiated into more or less separate groups by the MCA (Fig. 1).

Variety *globosa* is characterized by two unique alleles that occur in all sampled populations (Table 2). This variety is, likewise, monomorphic at the β-esterase locus (fixed for the most common allele), which is highly polymorphic in other varieties. The MCA (Fig. 1) clearly delineates *globosa* from other varieties. Comparisons of patterns of genetic vari-

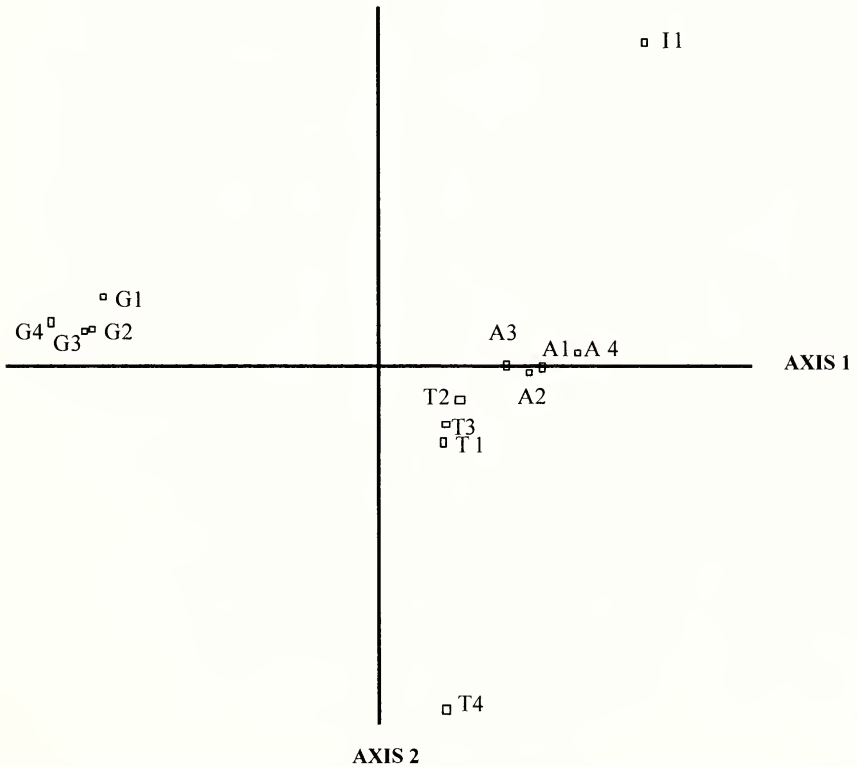

FIG. 1. Multiple Correspondence Analysis (MCA) of allele frequencies in *Isomeris arborea* populations. Variety *globosa* is clearly distinguishable by axis 1 which explains 62% of the variance. Axis 2 clusters the other varieties surveyed and explains 10% of the variance in the data. Population designations are given in Table 1.

ability in *globosa* with those in *arborea* and *angustata* show that the average genetic distance between *globosa* and these varieties is larger than that between those two varieties. The average pairwise F_{ST} value among *globosa* populations sampled is substantially less than that among *arborea* and *angustata* populations. Lewontin and Krakauer (1973) have argued that such a pattern might be expected if an entire section of a species complex originated from a relatively small isolated population that subsequently spread locally. We conclude, therefore, that Gitten's (1965) proposal that the ancestor of *globosa* was isolated from the other varieties and the globose form subsequently became fixed in this area is supported. Furthermore, the substantial genetic divergence detected between *globosa* and the other varieties as well as its clear morphological delineation leads us to the conclusion that *globosa* may deserve taxonomic placement as a subspecies of *Isomeris.*

Our results indicate that *I. arborea* is a species with moderate levels of genetic variation. For plant species exhibiting similar life-history characteristics (dicot, woody long-lived perennial, narrow geographic range, temperate distribution, outcrossing animal pollinated breeding system, early successional status) composite mean values from Hamrick and Godt (1990) for P, A, and H_E were 33.5%, 1.51, and 0.110 respectively. Corresponding values for *I. arborea* are substantially lower at 13.85%, 1.18 and 0.051. The gene diversity values we found in *I. arborea* are substantially different from that reported for similar species by Hamrick and Godt (1990, 1996): H_T = 0.276 vs. 0.320, H_S = 0.152 vs. 0.240, G_{ST} = 0.449 vs. 0.220 and D_{ST} = 0.124 vs. 0.07. The values we detected in *I. arborea* are, however, similar to those reported by Vanderpool et al. (1991) for the capers *Oxystylis lutea* and *Wislizenia refracta.* In addition to reporting a large number of unique alleles as found in this study, the apportionment of diversity among the populations they studied is quite similar to that reported here, ranging from 33 % for *Wislezenia refracta refracta* to 54 % for *Wislezenia refracta californica.* Furthermore, the average genetic diversity measures (P = 23%, A = 1.29, H_O = 0.075 and H_E = 0.073) reported are more congruent to those we report.

Whether the patchy distribution of *I. arborea* observed today is a remnant of a more continuous range in the past is difficult to assess since no information on *Isomeris* exists in the fossil record (Iltis 1957; Iltis personal communication). The significant degree of structure among varieties is indicative of considerable isolation. Metapopulation cohesiveness within a landscape depends upon dispersal. If the interaction between the dynamics of dispersal and disturbance (climatic/geologic change) reaches some critical level, populations within the matrix may become disconnected and processes within them unpredictable. The repeated contraction and expansion of habitat associated with the dynamic geologic and climatic history of the American Southwest may have played a major role in the distribution of genetic variation in *I. arborea.*

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